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(71) Applicant: **AMERSHAM INTERNATIONAL plc**
Amersham Place
Little Chalfont Buckinghamshire HP7 9NA(GB)

(72) Inventor: **Sadler, Peter John**
48 Park Crescent
Harrow Weald Middlesex HA3 6ES(GB)

(72) Inventor: **Harding, Charles Thomas**
Ash Trees Long Croft
Bovingdon Hertfordshire, HP3 0JL(GB)

(72) Inventor: **Kelly, James Duncan**
c/o Amersham International plc, White Lion Road
Amersham Bucks. HP7 9LL(GB)

(72) Inventor: **McEwan, Andrew Bruce**
4 Nightingale Court 54 Thrale Road
Streatham, SW16 1NY(GB)

(74) Representative: **Pennant, Pyers et al,**
Stevens, Hewlett & Perkins 5 Quality Court Chancery
Lane
London, WC2A 1HZ(GB)

(64) Contrast agent for NMR scanning.

(57) Complexes formed between a) an amino di- or poly-phosphonate in which phosphonate groups comprise separate carbon atoms and b) a paramagnetic metal ion such as Gd^{3+} , have calcified tissue seeking properties which make them useful as contrast agents for investigating bone metabolism by NMR scanning. Two preferred poly-phosphonates are ethylenediamine tetramethylphosphonate and meta-xylene-diamine tetramethylphosphonate.

CONTRAST AGENT FOR NMR SCANNING

This invention relates to a contrast agent for investigating calcified tissue by nuclear magnetic resonance (NMR) scanning. Contrast agents are used in NMR scanning to alter the T_1 and T_2 relaxation times of protons in their vicinity. Analysis of proton relaxation times is used to determine where in the body or other object being scanned the contrast agent has become located; and this in turn can provide information about the internal structure of the body. Calcified tissue contains relatively few protons as a result of low water content, and hence cannot be visualized in NMR scans. If a contrast agent could be caused to locate on or in calcified tissues and there to alter the T_1 or T_2 relaxation times of adjacent protons, it would be possible thereby to generate an NMR scanning image that might give valuable information about the state of the calcified tissues.

It is known that phosphates ($-O-PO_3H_2$) and phosphonates ($-C-PO_3H_2$) have an affinity for hydroxy-apatite crystals, and thus tend to locate in vivo in regions of bone metabolism. In "Use of Radiolabelled Compounds in Medicine" Chapter 34, G. Subramanian et al., compare and contrast the properties as bone agents of complexes of di- and poly-phosphates and phosphonates with the radioactive isotope Tc-99m. Kits for preparing radioactive bone agents by labelling some of these di and poly-phosphates and phosphonates with Tc-99m are available commercially. In addition, mention can be made of European Patent Specification 122813 which describes ethylene glycol-1,2-bis phosphonic acid and its complex with Tc-99m as a bone seeking agent.

It is known that Tc-99m agents intended to locate

in regions of bone metabolism can also locate in certain tumours, e.g. neuroblastoma, and other diseased tissues. Uptake of such agents in tumours is attributed to calcification in the tumours (Clinical
5 Nuclear Medicine, 11, 337-40, 1986). As used herein, the term calcified tissue includes bone and regions of bone metabolism, and also regions of calcification in tumours and other diseased tissues.

Technetium is a transition metal in group 7B of
10 the periodic table. It may reasonably be expected that other transition metal ions, including those with paramagnetic properties, will be capable of forming complexes with di- and poly-phosphonates.

EPA 133603 describes relaxation agents for
15 modifying the contrasts obtained in medical imaging by NMR. These relaxation agents are complexes of paramagnetic metal ions with a variety of organic complexants including polyamino polymethylphosphonates. But no practical data are provided, nor is there any
20 discussion of the biodistribution of the complexes.

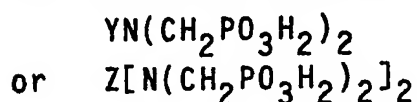
In German Patent Specification DOS 3129906, there are described complexes of various geminal di-phosphonates (that is to say, with two phosphonic acid groups attached to the same carbon atom) with ions of
25 elements of atomic numbers 57 to 70, 21 to 29, 42 or 44. These complexes are stated to be suitable for administration to patients for NMR diagnosis, but no animal results are given. In particular, no indication is given of the toxicity of the complexes or
30 of their solubility, which is important for administration. So far as biodistribution is concerned, it is merely stated that the complexes appear to be admirably suited for improved demarcation and location of lesions of the pancreas and liver and
35 of tumours and haemorrhages in the cranial region.

The possible use of poly-phosphonates for NMR bone scanning is not mentioned. In the light of the Schering disclosure, the following doubts remained:-

- 5 a) Di and poly-phosphonates are known to be toxic by reason of their ability to bind calcium ions. Similarly, most paramagnetic transition metal ions are toxic to a greater or lesser extent. Complexes of the two may be less toxic, but only provided that the complexes are stable in vivo. It was not predictable
10 whether this would be the case.
- b) The complexes can only be useful for skeletal imaging by NMR if they tend to locate in regions of bone tissue. It was not known whether this would be the case.
- 15 c) The complexes can only be useful for imaging by NMR if the complexed paramagnetic metal ion is nevertheless capable of altering the T_1 relaxation times of protons in its neighbourhood. It is known that complexing of the metal ions can reduce their
20 activity in this respect. It was not possible to tell whether paramagnetic metal ions which were sufficiently strongly complexed to avoid toxicity problems would nevertheless retain the ability to alter proton relaxation times.
- 25 d) The complexes can only be useful for skeletal imaging by NMR if they can be brought into solution in adequate concentrations at physiologically acceptable pH's. At least some of the complexes described are not sufficiently soluble.
- 30 According to the present invention, these doubts are resolved. The invention concerns a contrast agent for investigating calcified tissue by NMR scanning which agent comprises a complex formed between a) an amino di- or poly-phosphonate in which phosphonate
35 groups comprise different carbon atoms, and b) a

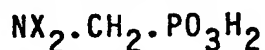
paramagnetic metal ion. The invention also concerns use of the complex for the preparation of the contrast agent. The invention also concerns the investigation of calcified tissue by NMR scanning following
5 introduction of the contrast agent into the body. Some of the complexes are new compounds and are claimed as such.

Preferred di- or poly-phosphonates contain the groups $-N(CH_2PO_3H_2)_2$ at one or more, generally one or
10 two, positions in the molecule. These phosphonates may have the formula:-



where each of Y and Z may be an aliphatic or aromatic
15 groups containing 1 to 12 carbon atoms which may also contain one or more hydroxyl, ether, carboxylate, sulphonate, amine or aminomethylphosphonate groups, or non-toxic salts thereof. Complexes containing aromatic groups are believed new.

20 A poly-phosphonate may for example have the general formula:-



where the two groups X are the same or different and each is $-CH_2PO_3H_2$ or $-(CH_2)_nN(CH_2PO_3H_2)_2$, and n is up
25 to 10, preferably 2 or 6, or a non-toxic salt thereof.

Examples of specific poly-phosphonates are nitrilo-trimethylphosphonate, hexamethylene diamine tetramethylphosphonate (HDTMP), ethylene diamine tetramethylphosphonate (EDTMP), diethylene triamine
30 pentamethylphosphonate (DTPMP), meta-xylene-diamine tetramethylphosphonate (MXDTMP), and non-toxic salts thereof.

Suitable paramagnetic metal ions are well known in the field and include those of the lanthanide elements
35 with atomic numbers 58 to 70, and those of the

transition metals with atomic numbers 21 to 29, 42 and 44. Preferred are Mn(II), Cu(II), Fe(II), Gd(III), Fe(III), Cr(III), Dy(III) and V(IV). Factors affecting the choice of metal ion are its paramagnetic properties, the stability and solubility of the metal ion phosphonate complex, its toxicity, and the extent to which the metal ion in the complex interacts with water so as to vary the proton relaxation times. The agents may also be used in a NMR chemical shift imaging technique.

The complexes are easily prepared, by mixing an aqueous solution of the chosen di- or poly-phosphonate with an aqueous solution of a salt of the chosen transition metal. Or a di- or poly-phosphonate may be added to an aqueous suspension of the transition metal carbonate. The precise structures of the complexes are not known with certainty, and may vary depending on the nature of reagents. The solutions may be buffered, generally at a pH in the range 4 to 10, and the mixture may be brought to a suitable pH for administration. Complex-formation takes place rapidly, and without the need for heating the mixed solutions.

Although equimolar amounts of the reagents may be used and give satisfactory results, in some cases it may be advantageous to use a higher molar concentration of the phosphate than of the metal ion. For example, in the case of the complex formed from EDTMP and Gd, it is preferred to use a molar ratio of EDTMP to Gd of at least 1.5. An upper limit for this ratio is set by the extent to which EDTMP is toxic and the amount of the complex to be administered. The toxic effects of excess EDTMP, or other phosphonates, are preferably countered by the incorporation of quantities of calcium within the formulation.

In order to investigate calcified tissue by NMR scanning, it may be necessary to administer, preferably by injection, an aqueous solution of the complex of from 0.5 to 250mM, preferably 1 to 50mM, concentration with respect to the paramagnetic metal ion. The volume for injection into a human subject is typically from 1 to 100ml. If the desired quantity would be hypertonic if presented in a small volume for injection, then it may be converted to organic salt form, for example to the N-methyl glucamine salt, in accordance with known procedures.

The Examples below are organized as follows.

Example 1 described the preparation of three complexes according to the invention and shows that these are capable of altering proton relaxation times (T_1 and T_2).

Example 2 describes the preparation of other complexes, and uses radioactive ^{153}Gd to demonstrate the extent to which these are bone-seeking in rats.

Example 3 describes the preparation of 12 further di- and poly-phosphonates.

Example 4 describes the preparation and proton T_1 relaxation properties of complexes from the phosphonates of Example 3.

Example 5 describes the preparation and proton T_1 relaxation properties of hydroxy apatite adducts of complexes of Example 4.

The results indicate that the complexes are bone-seeking in vivo, and act in bound form as effective contrast agents for investigating calcified tissue by NMR scanning.

Example 1

0.043g $\text{Gd}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ was mixed with 0.046g EDTMP in 5ml acetate buffer (pH 5.6). 1ml 1M NaOH was

added. To the clear solution a further 4ml of acetate buffer was added to make the total volume 10ml, (pH 5.6) containing 10mM concentration of a complex designated Gd.EDTMP.

5 Similarly by using HDTMP and DTPMP there were prepared aqueous solutions of complexes designated Gd.HDTMP and Gd.DTPMP respectively. Binding studies, via observation of T_1 and T_2 relaxation times in water have shown that the metal:ligand complexes are in a 1:1
10 ratio.

In relaxation studies of the three complexes, the T_1 results obtained are set out in Table 1.

Table 1

15

20	Concentration (mM)	T_1 (ms) at 200 MHz and 300°K		
		Gd.DTPMP	Gd.EDTMP	Gd.HDTMP
	5	23	18	30
	2.5	38	38	71
	1	45	87	142
25	0.5	144	168	284

The complexed Gd is clearly capable in all cases of altering T_1 relaxation times of protons in its neighbourhood.

30 Further studies were performed with Gd.EDTMP in a body scanner operating at 6 MHz. The T_1 relaxation time for water was determined as 1513ms, whereas the T_1 relaxation time for a 0.1mM Gd.EDTMP solution was determined as 722ms. This confirms that the
35 complexed Gd is capable of altering T_1 relaxation times

of protons under conditions likely to be used in practice.

Example 2

5 This example uses ^{153}Gd to compare the biodistribution properties of various complexes in rats.

10 Equal volumes of 1mM methylene diphosphonic acid and 0.5mM Gd^{3+} were used to prepare a complex designated Gd.MDP. The complex had poor solubility properties around physiological pH's.

Equal volumes of 10mM ethane-1-hydroxy-1,1-diphosphonic acid and 5mM Gd^{3+} were used to prepare a complex designated Gd.EHDP. But the pH of the mixture had to be kept above 7 to avoid precipitation.

15 Equal volumes of 10mM EDTMP and 5mM Gd^{3+} were used to prepare the complex Gd.EDTMP.

Using ^{153}Gd , a comparison was made of certain properties of aqueous "solutions" of

- 20
- $^{153}\text{GdCl}_3$
 - $^{153}\text{Gd.MDP}$ (0.5mM with respect to Gd)
 - $^{153}\text{Gd.EHDP}$ (5mM with respect to Gd)
 - $^{153}\text{Gd.EDTMP}$ (5mM with respect to Gd)

25 The results are set out in Table 2. The biodistribution data there reported were obtained by means of an IV study in male rats which were sacrificed two hours post injection. % activity per organ is given, together with ratios of bone to organ.

30

35

Table 2

% Activity	$^{153}\text{GdCl}_3$	$^{153}\text{Gd.MDP}$	$^{153}\text{Gd.EHDP}$	$^{153}\text{Gd.EDTMP}$
Bone	38.6	3.1	22.64	45.2
Muscle	4.0	0.3	13.8	1.1
Blood	3.7	0.1	21.45	0.6
Kidneys	2.5	0.3	1.05	0.4
Bladder & Urine	12.5	1.0	24.0	45.8
Lung	0.6	0.4	1.55	0.1
Liver & Spleen	32.5	93.9	11.29	1.4
Stomach & Gut	3.9	0.0	4.21	1.9
Carcass	1.8	0.7	0.01	3.5
Injection Site	3.4	0.5	2.91	2.1
RATIOS				
Bone/Muscle	83	80	14.07	491.2
Bone/Blood	12.2	33.2	1.24	173.0
Bone/Liver & Spleen	1.1	0.0	2.04	35.2

These results show that the complexes Gd.MDP and Gd.EHDP are not useful as bone scanning agents. On the other hand, the invention complex Gd.EDTMP is shown to be a good bone-seeking agent, and a good T_1 relaxation agent for water.

It is known that Ca^{2+} can bind to di- and polyphosphonates, and that this is one reason why these materials are toxic. It is also known that dimethylglucamine can be used to solubilize complexes

of this kind. Checks on whether the bone uptake of the two complexes Gd.EDTMP and Gd.EHDP was affected by the addition (separately) to their aqueous solutions of Ca^{2+} and dimethylglucamine; showed that neither addition had a significant effect.

Example 3

The phosphonates listed below were synthesised by a direct Mannich type reaction using an amine, formaldehyde and phosphorous acid. The basic reaction is given in the paper by Moedritzer (JCS 1966 vol. 31 p 1603). The reaction is seen by ^{31}P NMR to proceed quantitatively and isolation of the product was generally achieved by filtration and washing of the precipitate which forms during the reaction. In some cases isolation was achieved by concentration of the reaction mixture and in others by addition of ethanol to the concentrated reaction mixture. The method used is indicated below. The general reaction scheme is to heat the solution of the amine, phosphorous acid and concentrated HCl to reflux temperature. 100% excess of aqueous formaldehyde solution was then added over 1 hour. The reaction mixture is then refluxed for a further 1 hour and the product isolated.

Example

para-xylene diamine tetramethylene phosphonate
41g H_3PO_4 were dissolved in 100ml of H_2O /concentrated HCl (50:50 v.v) and 17g p-xylene diamine were added. The solution was brought to reflux and 80ml formaldehyde solution 37% w/v were added over 1 hour. The solution was refluxed for a further hour during which precipitation of the product occurred. The solution was allowed to cool and the product filtered and washed with water, ethanol and ether, and dried at 100°C .

Yield 45.8g

m.p. 249-251°C

Calculated for $C_{12}H_{26}N_2O_{12}P_4$

C 28.12 H4.69 N5.47 P24.21

5 Found C28.09 H4.79 N5.49 P24.04

Ligands Synthesised

Amino ethanol dimethylene phosphonate - AEDMP

Glycine dimethylene phosphonate - GDMP

Gamma amino butyric acid dimethylene phosphonate -

10 GABADMP

6-amino hexanoic acid dimethylene phosphonate - AHADMP

Meta-xylene diamine tetramethylene phosphonate - MXDTMP

Para-xylene diamine tetramethylene phosphonate -

PXDTMP

15 1,3-diamino-2-hydroxy-propane tetramethylene
phosphonate - DHPTMP

3,3'-diaminopropyl-N-methyl dipropylamine
tetramethylene phosphonate - DPNDPTMD

4,9-Dioxa-1,12-dodecane-di-amine tetramethylene
20 phosphonate DDDATMP

Di-propylene triamine pentamethylene phosphonate -
DPTMP

Triethylene tetramine hexamethylene phosphonate - TTHMP

N,N'-bis (3 amine propyl) ethylene diamine

25 hexamethylene phosphonate - BAPEDHMP

	<u>Ligand</u>	<u>m.p.</u>	<u>Yield</u>	<u>Isolation</u>	<u>Form</u>
	AEDMP	235-237°C	40.1%	C	White crystals
	GDMP	200-201°C	60.8%	B	White crystals
30	GABADMP	202-204°C	63.3%	B	White crystals
	AHADMP	192-194°C	84.4%	A	White crystals
	MXDTMP	238-239°C	74.2%	A	White crystals
	PXDTMP	249-250°C	76.5%	A	White crystals
	DHPTMP	272-275°C	60.5%	C	White crystals
35	DPNDPT	228-230°C	65.1%	C	White crystals

	<u>Ligand</u>	<u>m.p.</u>	<u>Yield</u>	<u>Isolation</u>	<u>Form</u>
	DDDATMP	145-147°C	70.3%	C	White crystals
	DPTPMP	195-299°C	58.8%	C	White crystals
	TTMMP	206-208°C	42.8%	B	Off white crystals
5	BAPEDHMP	192-200°C	85.0%	C	White crystals

Isolation

- A - Crystallised out of reaction mixture
 B - Crystallised out after reducing volume
 10 C - Crystallised out after reducing volume and adding ethanol

All products isolated as white/off white powders regular shapes under microscope.

Example 4

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1. The preparation of copper, nickel, manganese and cobalt complexes was achieved by heating the ligands, in suspension, with the metal carbonate.

Example

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- Preparation of manganese metaxylene diamine tetramethylene phosphonate.

2.6g MXDTMP and 0.56g of MnCO_3 were heated together in 200ml H_2O for 1 hour. The solvent was then removed under reduced pressure to produce a free
 25 flowing white powder.

Yield 2.65g Calculated for $\text{C}_{12}\text{H}_{28}\text{N}_2\text{O}_{12}\text{P}_4$ C23.08
 H4.29 N4.62 P20.49 96% recovery Found C23.08
 H4.13 N4.72 P19.51

2. Complexes with metals such as chromium and
 30 gadolinium were synthesised by mixing equal molar quantities of ligand and metal salt. The methods differ in exact technique.

$\text{Gd}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ is added slowly to a solution of the sodium salt of the ligand at pH 7.0. There is
 35 precipitation but this is redissolved upon adding NaOH(dilute). Any suitable strength Gd phosphonate

solution can be made in this way. Ligands containing less than four amino phosphonates were added at a concentration twice that of the metal with higher numbers of $-\text{CH}_2\text{PO}_3\text{H}_2$ groups the metal ligand ratio is 1:1. Chromium (Cr) complexes were made by boiling equimolar amounts of ligand and $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ or in some cases ligand present in 2:1 excess as above. The reaction was seen to occur by noting the colour change blue to green. The mixtures were refluxed for 1-2 hours. The pale green precipitate was redissolved by addition of dilute NaOH to give a pH of about 7.0.

Relaxation Data 1

Relaxation measurements have been made on the phosphonate complexes at 200MHz and 10MHz a clinically relevant field strength.

Chromium		200MHz 25°C 1 millimolar concentration
Complexes		T_1
Water Standard		3.3s
Cr(AEDMP) ₂		1.13s
20	Cr(GDMP) ₂	0.89s
	Cr(GABADMP) ₂	0.91s
	Cr(AHADMP) ₂	1.04s
	CrMXDTMP	0.55s

25	Gadolinium Complex 200MHz 25°C 1 millimolar concentration	
	GdMXDTMP	0.064s

Relaxation Data 2

Manganese Complexes		200MHz 25°C 1 millimolar concentration
		T_1
30	Water Standard	3.3s
	Mn(AEDMP) ₂	0.007s
	Mn(GDMP) ₂	0.160s
	Mn(GABADMP) ₂	0.072s
35	Mn(AHADMP) ₂	0.125s
	MnMXDTMP	0.097s

The aromatic ligand MXDTMP when complexed to metal results in efficient T_1 relaxations. The lines obtained at 200MHz are sharp which implies less effect on T_2 relaxation. This would result in efficient T_1 relaxation but without loss of signal intensity due to line broadening.

Relaxation Data 3

Relaxation measurements have been made at 10MHz at 37°C for the complexes of MXDTMP at 10mM concentration.

	T_1	T_2
CrMXDTMP	160 ms	130 ms
MnMXDTMP	10 ms	9 ms
GdMXDTMP	10 ms	7 ms

At 10 MHz 37°C MXDTMP gives better relaxation than EDTMP.

	T_1	T_2	T_1/T_2
MnMXDTMP	10 ms	9 ms	1.1
MnEDTMP	27 ms	24 ms	1.1

10 millimolar solutions

Example 5

Binding to Hydroxy Apatite.

1ml of hydroxy apatite solution 250mg/ml in water was used for all the binding assays to be described.

1. Binding of Ligands

The solid from 1 ml of hydroxy apatite solution was isolated by centrifugation and the supernatant removed. 0.4ml of a 0.1M solution of ligand at pH 7.5 ± 0.1 were then added and the solid resuspended. The solid was once more spun down and the supernatant analysed by ^{31}P NMR. This enabled the amount of bound phosphonate to be analysed.

2. Binding of Complexes

The procedure above was followed but this time 0.4ml of 50mM metal complex solution was added and the solid resuspended. The supernatant was analysed by T_1

using T_1 relaxation measurements and the amount bound to hydroxy apatite calculated.

3. Relaxation of Hydroxy Apatite-Complex Adducts

The binding studies above produced hydroxy apatite with phosphonate complexes bound. These solids were resuspended at 10mg/ml and the T_1 and T_2 relaxation times measured at 10 MHz and 37°C.

Binding of Ligands to Hydroxy Apatite ^{31}P Study

	<u>Ligand</u>	<u>% Bound to HAP</u>
10	AEDMP	50.9
	GDMP	88.2
	GABADMP	77.8
	AHADMP	89.7
	EDTMP	89.4
15	BDTMP	75.7
	MDTMP	85.5
	ODTMP	84.9
	MXDTMP	84.1
	PXDTMP	88.0

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Binding of Chromium Complexes to Hydroxy Apatite

	<u>Complex</u>	<u>% Bound</u>
	None (No T_1 change)	None
	Cr(AEDMP) ₂	96%
25	Cr(GDMP) ₂	80%
	Cr(GABADMP) ₂	90%
	Cr(AHADMP) ₂	96%
	CrMXDTMP	96%

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Relaxation of Hydroxy Apatite-Complex Adducts

Complex 10mg/ml	T_1 10MHz 37°C	T_2 10MHz 37°C
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	None HAP only	2.50s	0.97s
5	Cr(AEDMP) ₂	1.03s	0.53s
	Cr(GDMP) ₂	0.97s	0.54s
	Cr(GABADMP) ₂	1.39s	0.67s
	Cr(AHADMP) ₂	1.17s	0.55s
	CrMXDTMP	0.98s	0.56s
10	Mn(AEDMP) ₂	0.14s	0.12s
	Mn(GDMP) ₂	0.24s	0.24s
	Mn(GABADMP) ₂	0.24s	0.16s
	Mn(AHADMP) ₂	0.26s	0.18s
	MnEDTMP	0.23s	0.13s
15	MnBDTMP	0.19s	0.13s
	MnMDTMP	0.08s	0.06s
	MnMXDTMP	0.13s	0.11s
	GdMXDTMP	0.06s	0.06s

The ^{31}P study shows few differences between ligand all except (AEDMP) binding about 80% to hydroxy apatite and the figures are not significant when one realises the large errors in quantitation in NMR.

The binding of metal complexes do show differences and this experiment is such that these figures are significant. Small changes in ligand structure give different binding and the model separates the complexes on adsorption characteristics.

The last set of results shows that the complexes once bound still give relaxation of water molecules. The reduction is very significant and indicates the utility of these compounds in vivo. The differences in T_1/T_2 for different complexes may be useful to decide which complexes would give rise to good T_1 relaxation changes with the least loss of signal intensity.

This hydroxy apatite experiment indicates that the complexes will bind to calcified tissue in vivo, and will act in bound form as effective contrast agents for investigating calcified tissue by NMR screening. But
5 the hydroxy apatite-complex adducts are likely to have utility of their own. After oral administration, they are likely to permit NMR imaging of the gastrointestinal tract. For this purpose the adducts compare favourably with those described in EPA 183760.

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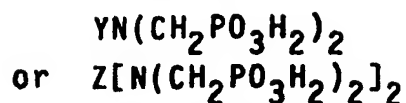
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CLAIMS

1. Use, for the preparation of a contrast agent for investigating calcified tissue by NMR scanning, of a complex formed between a) an amino di- or poly-phosphonate in which phosphonate groups comprise
5 different carbon atoms, and b) a paramagnetic metal ion.
2. Use as claimed in claim 1, wherein the di- or poly-phosphonate contains the group $-N(CH_2PO_3H_2)_2$.
- 10 3. Use as claimed in claim 1 or claim 2, wherein the poly-phosphonate has the general formula $NX_2CH_2PO_3H_2$, where the two groups X are the same or different and each is $-CH_2PO_3H_2$ or $-(CH_2)_nN(CH_2PO_3H_2)_2$, and n is up to 10, or a non-toxic salt thereof.
- 15 4. Use as claimed in claim 3, wherein the poly-phosphonate is ethylenediamine tetramethylphosphonate.
5. Use as claimed in claim 1 or claim 2, wherein the poly-phosphonate is meta-xylene-diamine tetramethylphosphonate.
- 20 6. Use as claimed in any one of claims 1 to 5, wherein the paramagnetic metal ion is Gd(III) or Fe(III).
7. Use as claimed in claim 6, wherein the complex is formed from a) ethylenediamine tetramethylphosphonate and b) Gd(III) in a molar ratio of a) to b) of at
25 least 1.5.
8. Use as claimed in claim 7, wherein the complex is present in solution at a concentration of from 1 to 20mM with respect to Gd.
- 30 9. An adduct with hydroxy apatite of a complex formed between a) an amino di- or poly-phosphonate in which phosphonate groups comprise different carbon atoms, and b) a paramagnetic metal ion.
10. A complex formed between a) a di- or poly-

phosphonate having the formula



5 where each of Y and Z is an aliphatic or aromatic group containing 1 to 12 carbon atoms including at least one continuous chain of 3 or more carbon atoms, which group may also contain one or more hydroxyl, ether,

carboxylate, sulphonate, amine or
10 aminomethylphosphonate groups, or a non-toxic salt thereof, and b) a paramagnetic metal ion.

11. A complex as claimed in claim 10, wherein the paramagnetic metal is Gd^{3+} .

12. A complex formed from meta-xylene-diamine
15 tetramethylphosphonic acid or a non-toxic salt thereof, and Gd^{3+} .

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